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PRINCIPAL INVESTIGATOR:
David L. Brody, MD PhD

CONTRACTING ORGANIZATION:
Washington University, St Louis MO 63110,

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14. ABSTRACT The objective of the project is to test two advanced MRI methods, DTI and resting-state fMRI correlation analysis, in military TBI patients acutely after injury and correlate findings with TBI-related clinical outcomes 6-12 months later. An additional objective is to test the interaction of candidate genetic vulnerability factors with patterns of injury. These combined methods may add clinically useful predictive information following traumatic brain injury that could be of assistance in standardizing diagnostic criteria for TBI, making return-to-duty triage decisions, guiding post-injury rehabilitation, and developing novel therapeutics. The overarching hypothesis is that traumatic axonal injury, interacting with genetic vulnerability factors, is a principal cause of impaired brain function following blast-related and non-blast-related TBI. The study is a prospective longitudinal study with subject enrollment and initial evaluation at Landstuhl Regional Medical Center in Landstuhl Germany. Follow-up evaluations are performed at Washington University in St Louis. Starting this year, additional subjects have been enrolled at 2 sites in Afghanistan To date, 223 subjects have been enrolled at LRMC and 230 subjects have been enrolled in Afghanistan. 145 complete follow-up evaluations have been performed. There have been no adverse events.					
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Introduction

The objective of the project is to test two advanced MRI methods, DTI and resting-state fMRI correlation analysis, in military TBI patients acutely after injury and correlate findings with TBI-related clinical outcomes 6-12 months later. The interaction of candidate genetic vulnerability factors with patterns of injury will be evaluated. These combined methods may add clinically useful predictive information following traumatic brain injury that could be of assistance in standardizing diagnostic criteria for TBI, making return-to-duty triage decisions, guiding post-injury rehabilitation, and developing novel therapeutics.

The overarching hypothesis is that traumatic axonal injury, interacting with genetic vulnerability factors, is a principal cause of impaired brain function following blast-related and non-blast-related TBI.

The specific aims of the proposal are as follows:

- Aim 1) To determine whether DTI and fcMRI will noninvasively reveal abnormalities that are not present on CT or conventional MRI acutely following blast-related and non-blast-related TBI. For this aim, a total of 200 participants with TBI, 100 with blast-related injuries and 100 with non-blast-related injuries, will be recruited at LPMC over a 2 year period.
- Aim 2) To assess the frequency of clinically occult traumatic axonal injury resulting from blast and non-blast mechanisms that is detectable using DTI, fcMRI, and conventional MRI. For this aim, a total of 200 participants without TBI but with other injuries will be recruited at LPMC during the same 2 year period: 100 with blast-related injuries and 100 with non-blast-related injuries.
- Aim 3) To use DTI and fcMRI to clarify the principal similarities and differences between blast-related TBI and TBI due to other mechanisms (e.g. motor vehicle accidents, falls, and direct blows to the head). This will be analyzed using the same 4 groups of participants described above in aims 1 and 2.
- Aim 4) To test the hypothesis that specific pattern of injuries detected with these methods will predict specific longer-term neurological and neuropsychological deficits. We will collect detailed clinical information on TBI-related outcomes 6-12 months after injury at Washington University. This will include standardized neurobehavioral assessments, neuropsychological testing, and structured interviews for depression and post-traumatic stress disorder. Several pre-specified hypotheses based on known brain anatomical-clinical correlations will be tested. Also, exploratory approaches will be used as the structural bases for many post-traumatic deficits and disorders are not well understood.
- Aim 5) To test the hypothesis that specific genetic factors interact with patterns of injuries to further increase the risk of specific neurological, neuropsychological, and psychiatric deficits and disorders. At follow-up, blood will be drawn for genetic testing. Genetic testing will be performed for *GABRA2* and *FKBP5* polymorphisms associated with PTSD, *5-HTTLPR* polymorphisms associated with increased risk of depression and PTSD following stressors, and *APOE* and *IL1 β* genotypes associated with poor recovery from TBI.

Since the last report, we have obtained additional funding through DARPA to support analysis of DTI data acquired in Afghanistan using MRI scanners installed in that country at 3 US military bases. The hypothesis guiding the studies in Afghanistan is that acute DTI abnormalities after blast-related TBI will reveal axon injury not apparent at later times, and help guide early return-to-duty decisions.

Body

During the second year of the project, we have made substantial progress towards these aims. We have worked closely with clinical coordinators and MRI technologists at Landstuhl regional medical center (LRMC). We have enrolled a total of 223 subjects at LRMC and 230 subjects in Afghanistan as of Sept, 2012 (**Table 1**).

Group	Number of Subjects Enrolled
LRMC A: Blast exposed active-duty US military controls (no TBI)	24
LRMC B: Blast-related active-duty US military TBI subjects	68
LRMC C: Non-blast-exposed active-duty US military controls (no TBI)	93
LRMC D: Non-blast-related active-duty US military TBI subjects	38
AFG E: Blast-related active-duty US military acute TBI subjects	115
AFG F: Control active-duty US military subjects with other injuries (no TBI)	115

There have been no adverse events. All acute DTI, fMRI and conventional MRI scans performed using the new Siemens 3T scanner at LRMC have been of good quality. We have been allotted 1-2 scans per working day on the scanner. We have successfully transferred MRI data and clinical information to Washington University without breaches of HIPAA regulations or other disclosure of confidential information using our Medweb server.

Dr. David Zonies has served as site PI at LRMC.

Ms. Tess Stewart, RN has continued at LRMC to serve as a research coordinator for our study. In addition Ms Elizabeth Kasten, RN has joined team as a research coordinator. Dr. Mac Donald has trained both coordinators.

The PI and Dr. Mac Donald performed a site visit at LRMC in June, 2012. No concerns were raised.

Dr. Octavian Adam is the PI for the Afghanistan study. Our role at WashU is to perform the DTI analysis, perform 6-12 month clinical evaluations, genetic analyses, and repeat the scans at 6-12 months. This work is supported by additional funds from DARPA transferred to CDMRP and combined with the current grant funds.

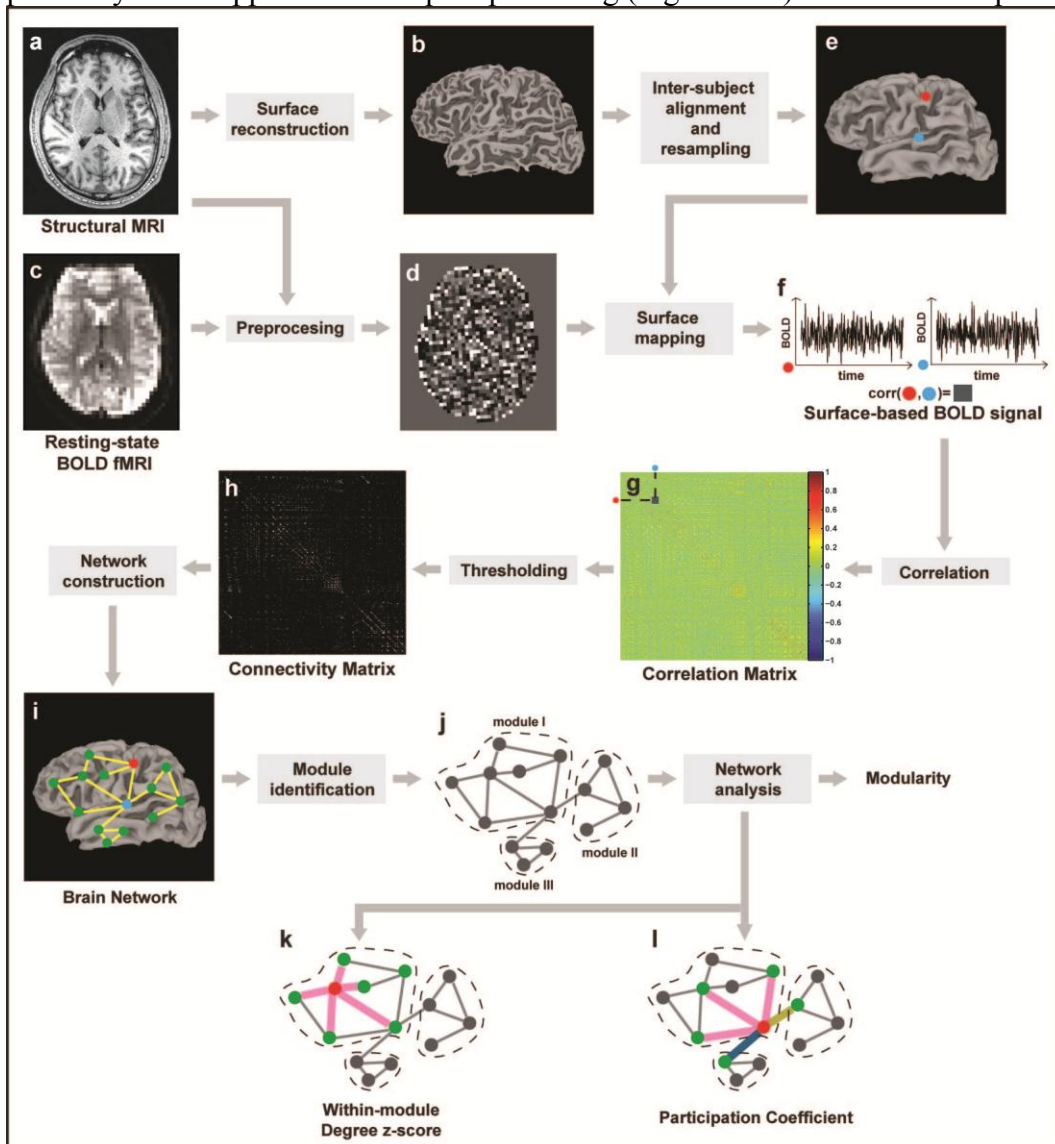
At Washington University, our clinical coordinators have performed telephone-based monthly clinical assessments and scheduled in-person follow-up evaluations in St Louis. We have continued to work with our excellent team of psychometricians and clinical psychologists to perform the in-person neuropsychological and psychiatric evaluations. We have performed 145 of these in-person evaluations as of September 2012. Others have been scheduled through 2013. There have been no complications arising from the evaluations. All subjects have been able to complete transportation in and out of St. Louis. No adverse effects of repeat MRI scans, neuropsychological testing, and psychiatric evaluations have been observed.

Since switching from blood to saliva for the genetics portion of the study, we have had 100% success at obtaining consent for the genetic studies. We have obtained IRB approval to re-contact subjects who have already been evaluated but declined genetics assessments and asked them to consider sending a saliva sample by Fedex. 27 subjects agreed and send saliva by Fedex.

We have begun using an automated DTI analysis method called DTIStudio. This had yielded excellent and consistent results when compared to hand-drawn region-of-interest analyses. It has substantial advantages in terms of time savings, and covers 130 regions of the brain rather than the 12 regions we were previously analyzing.

We have a large, stable excel-based database with all of the data entered and up-to-date.

Dr. Kihwan Han, a post-doctoral fellow in the group, has been exploring new methods for analyzing the fcMRI data. His graph theory based-approaches are quite promising (Figures 1-2) and a manuscript is currently in



preparation.

Figure 1. An illustration of the analysis procedure. For each subject, with volumetric structural MRI data (a), cortical surface (b) was reconstructed. Subsequently, the surface underwent the inter-subject alignment and spatial resampling close to the spatial resolution of resting-state BOLD fMRI (c) to allow surface-based, node-by-node cross-subject analyses. The preprocessed resting-state BOLD fMRI data (d) were converted to surface-based BOLD signal data (f) aligned to the individual cortical surface (e). BOLD fluctuation correlation coefficients between every pair of nodes in the brain (e.g., the gray square from red and cyan nodes in (e)) were obtained to yield a correlation matrix (g). A connectivity matrix (h) was derived by thresholding the correlation matrix, and a brain network (i) was constructed. In this illustration, yellow lines indicate connection between nodes. With the identified modules (three modules delineated by dashed lines in this example) in (j), modularity, within-module degree z-score (e.g., five magenta lines for the red-colored node in (k)) and participation coefficient (e.g., the distribution of magenta, cyan and olive lines for the red-colored node in (l)) were obtained for each node.

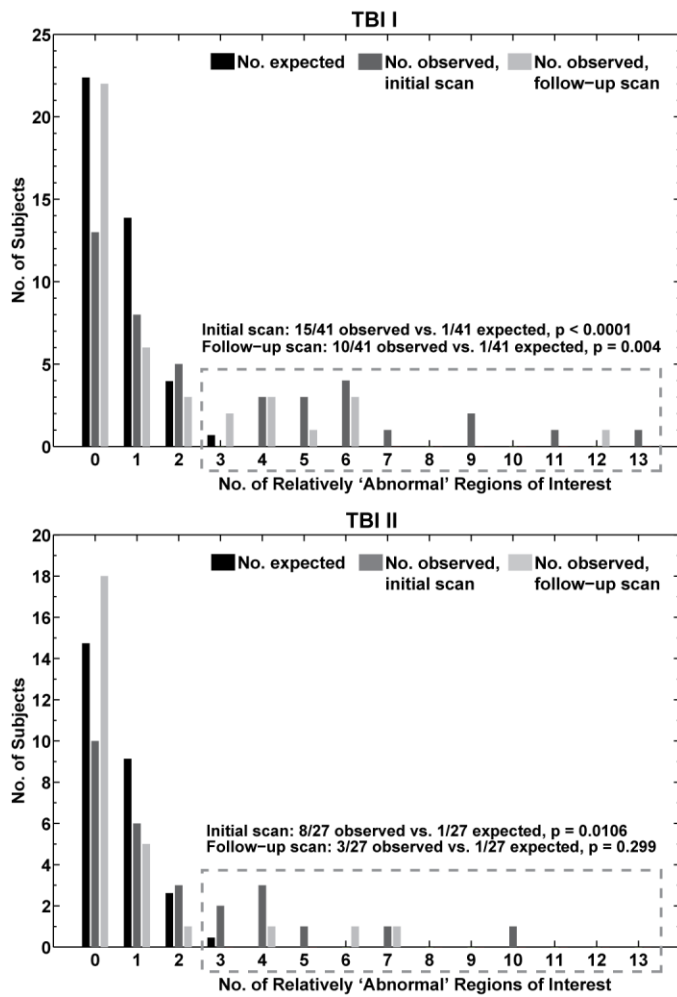


Figure 2. Bar graphs for observed and expected 'abnormal' ROIs in the patients with TBI relative to the controls. The distribution of expected relatively 'abnormal' ROIs was calculated from the binomial distribution with the probability that one region deems 'abnormal' by chance (0.0455; the probability that participation coefficient for a TBI patient falls outside two standard deviations from the mean of the controls). The p -values were obtained by the chi-square test.

We have begun analysis of genetic polymorphisms in an initial cohort of 134 subjects, combining across subjects enrolled in this study and in our previous 2007-funded LRMC study. In initial analyses of 16 single nucleotide polymorphisms, we have found that an intronic polymorphisms in the FKBP5 gene is associated with less severe symptoms of post-traumatic stress disorder in US military personnel with traumatic brain injury, but no change or slightly more severe symptoms in those with other injuries (Figure 3). We hypothesize that effects of specific genetic polymorphisms may be even clearer once injured US military personnel with white matter injuries that specifically affect emotional regulation circuits are selectively analyzed. The FKBP5 gene is a known regulator of glucocorticoid receptor sensitivity in the peripheral immune system but its function in the brain is not understood. The intronic polymorphisms affect expression levels in vitro, but their function in vivo is unknown. Future investigations in animal models will be required to determine the mechanistic basis of this effect.

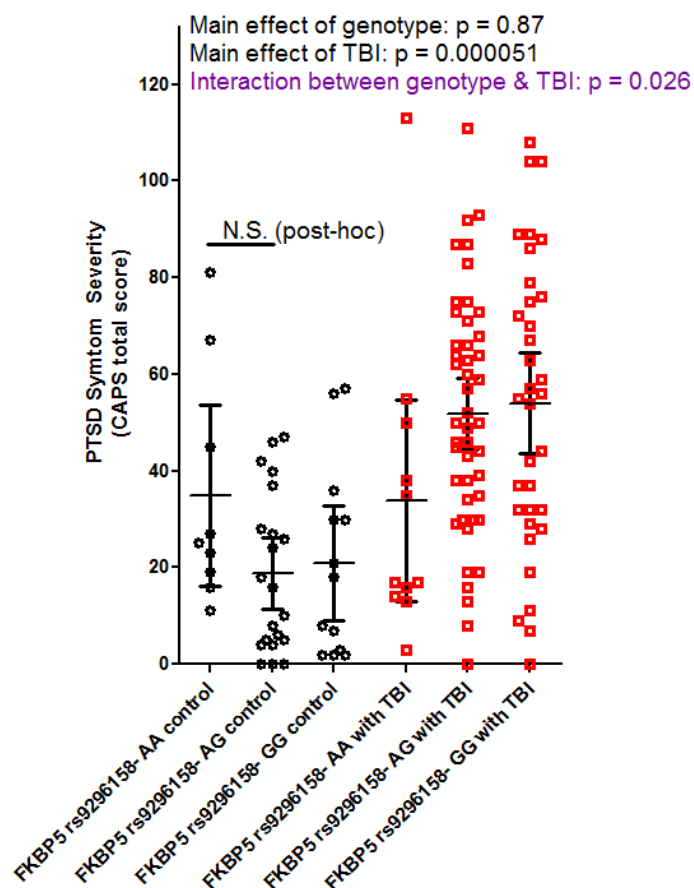


Fig. 3: A genetic polymorphism affecting the severity of post-traumatic stress disorder 6-12 months after injury in US military personnel with traumatic brain injury (red) or with other injuries sustained during deployment without (black).

Two –way ANOVA: main effect of injury $p=0.000051$, main effect of genotype $p=0.87$, interaction $p=0.026$. (Mac Donald and Brody, unpublished data)

This gene, FKBP, is quite interesting in that it has a strong biological plausibility. It has been implicated as a chaperone for glucocorticoid receptor signaling (Fig 4). In lymphocytes, it reduces the affinity of the receptor for cortisol and thereby may affect response to stress. Its role in the brain has yet to be determined. Furthermore, it is a validated drug target; the FDA approved immunosuppressant FK506 acts by binding to this family of proteins.

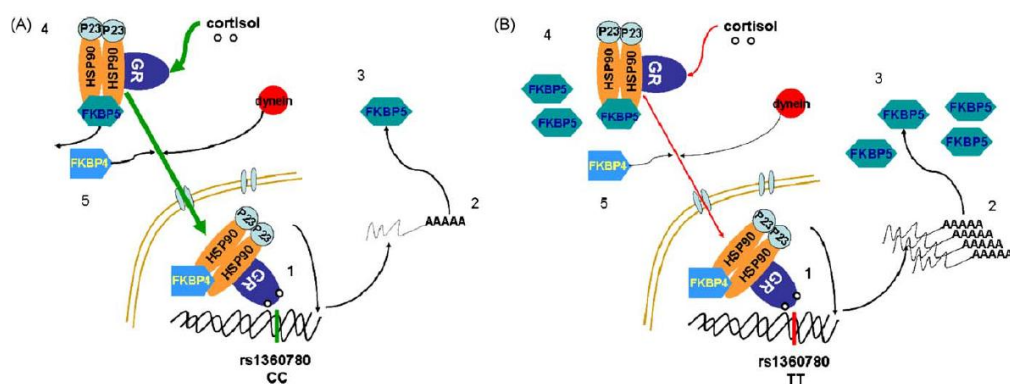
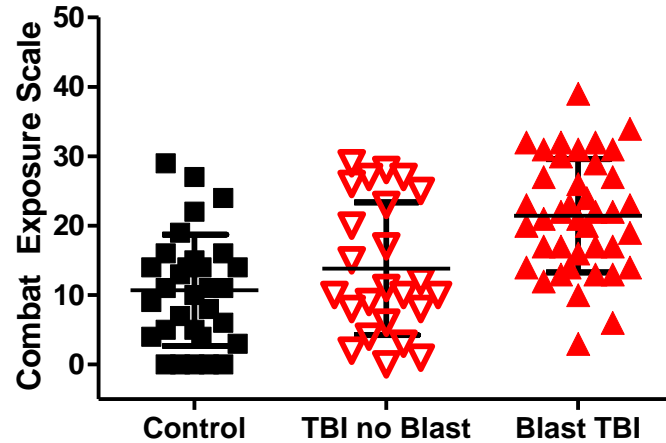


Fig. 4: Hypothesized mechanism of FKBP5 polymorphism effect. From Binder Psychoneuroendocrinology 2009

In response to the question raised regarding whether increased PTSD symptom severity could be the result of increased combat exposure rather than TBI, we directly addressed the self-reported combat exposure scale scores in a subset of participants. This was found to be higher in blast-related TBI subjects than in other groups (Fig 5).

Fig. 5 Combat Exposure Scale. The blast-TBI group has a significantly higher combat exposure score in comparison to control ($p < 0.0001$), 1-way ANOVA followed by Bonferroni correction for multiple comparisons. (Mac Donald and Brody, unpublished data)



However, there was no correlation between combat exposure score (CES) and PTSD severity (CAPS severity score) in the blast-related TBI subjects (Fig 6). Thus, combat exposure severity does not appear to be the driving factor underlying increased PTSD severity in the blast-TBI group. We hypothesize that injury to specific brain circuits involved in emotional resiliency and extinction learning are instead responsible for the increased PTSD severity following blast-related TBI. This hypothesis will be rigorously tested in the data sets acquired for this study.

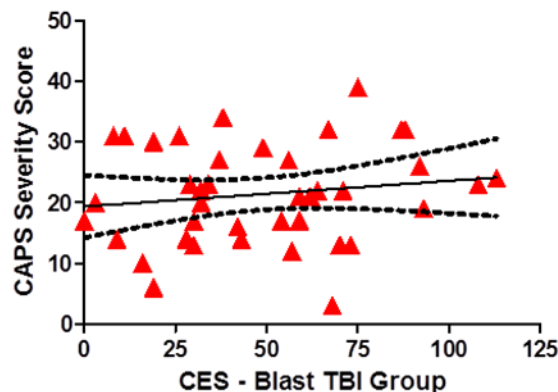


Fig. 6 Relationship between CAPS PTSD Severity and Combat Exposure in Blast-Related TBI Subjects. No correlation was observed between the PTSD severity as measured by the CAPS and the intensity of combat exposure in blast-related TBI subjects ($r^2 = 0.022$, $p = 0.3503$). Even though the blast-TBI group has a significantly higher level of combat exposure than the non-blast TBI or controls, this more intense exposure did not appear to drive PTSD severity in these subjects.

Key Research Accomplishments:

- Enrollment of 223 subjects at LRMC from all 4 planned groups and 230 subjects in Afghanistan.
- Completion of 145 in-person follow-up evaluations in St Louis.
- Initial Genetic analyses
- Initial fMRI analyses

Reportable Outcomes from the Current Project:

None.

Abstracts and Presentations:

The PI and Dr. Mac Donald presented aspects of the results at several meetings and seminars:

1. 2012 ATACCC meeting.
2. 2012 Research Seminar at Baylor College of Medicine
3. 2012 National Neurotrauma Society Meeting
4. 2012 Research Seminar at Wayne State
5. 2012 Research Seminar at Loma Linda
6. 2012 Research Seminar at University of Missouri, Columbia
7. 2012 Neurology Grand Rounds at the University of Pennsylvania
8. 2011 Society for Neuroscience Meeting

Conclusion:

The study is proceeding according to the proposed plan. Enrollment has been slower than anticipated at LRMC, but we have enrolled additional subjects in Afghanistan in collaboration with Dr. Octavian Adam. No major roadblocks have been encountered, and several exciting new directions are emerging. We are particularly intrigued by the genetic association between FKBP5 polymorphisms and resilience to PTSD following TBI. We will continue to focus on recruiting subjects from the blast-related TBI group during the next recruiting period.

References:

1. Binder, E. B. (2009). "The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders." Psychoneuroendocrinology **34 Suppl 1**: S186-195. <http://www.ncbi.nlm.nih.gov/pubmed/19560279>

Appendices: None.